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חוק הפטנטים, תשכ"ז - 1967  
Patent Law, 5727 - 1967  
בקשה לפטנט  
Application for Patent

אנ', (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)

Ben-Gurion University of the Negev  
Research and Development Authority  
P.O.Box 653, Beer Sheva 84105, Israel

1) אוניברסיטת בן-גוריון בנגב  
הרשות למחקר ופיתוח  
ת.ד. 653, באר שבע 84105

Mor Research Applications, Ltd.  
P.O. Box 7590, Pctach Tikva 49170

2) מור ישום מחקרים בע"מ  
ת.ד. 7590, פתח-תקוה 49170

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הרכבים ושיטות לטיפול ומניעת נקרוסיס

(בעברית)  
(Hebrew)

COMPOSITIONS AND METHODS FOR TREATING AND PREVENTING  
NECROSIS

(באנגלית)  
(English)

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| *מבקשת פטנט<br>from Application  |  | מספר/סימן<br>Number/Mark           | תאריך<br>Date | מדינת האיגוד<br>Convention Country |
| מס' _____<br>dated _____ מיום _____  |  |                                    |               |                                    |
| *פיוי כח: כללי / מיוחד - רצוף בזה / עווד יוגש<br>P.O.A.: general / individual - attached / -to be filed later<br>הוגש-בענין _____<br>filed-in case _____ |  |                                    |               |                                    |
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היום 25 בחודש מרץ שנה 2002  
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חתימת המבקש  
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Pyernik Rutman

By:   
Attorneys for Applicant

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הרכבים ושיטות לטיפול ומניעת נקרוטיס

COMPOSITIONS AND METHODS FOR TREATING AND PREVENTING  
NECROSIS

**Compositions and Methods for Treating and  
Preventing Necrosis**

**Field of the Invention**

The present invention relates to methods and compositions for treating and preventing cell necrosis. More specifically, the methods and compositions of the present invention prevent or treat necrosis by means of inhibiting the expression, activation or activity of intracellular elastase, or by over expression of one or more endogenous inhibitors of said intracellular elastase.

**Background of the Invention**

Elastase is a serine protease that catalyses the degradation of proteins, including elastin, a major structural protein of mammalian connective tissue. The art has suggested that the inhibition of elastase may be effective in the treatment of various conditions and diseases.

For example, US 4,683,241 indicates that elastase is believed to play an important role in the etiology of inflammatory connective tissue diseases. This patent discloses a class of phenolic esters exhibiting elastase inhibitory action.

US 5,216,022 discloses the use of aromatic esters of phenylenedialkanoates as inhibitors of human neutrophil elastase (also known as leukocyte elastase), for treating numerous neutrophil elastase-mediated conditions.

US 6,159,938 indicates that the inhibition of endogenous vascular elastase may be effective in treating pulmonary vascular disease and other related conditions.

Necrosis is the relatively uncontrolled process of cell death following perturbation to the cellular environment, resulting in cell rupture. Necrosis may be treated by the use of high pressure oxygen.

#### Summary of the Invention

The inventors have unexpectedly found that intracellular elastase is involved in necrotic cell death, and that the inhibition of said enzyme within the affected cells may serve as an effective tool for treating and/or preventing cell necrosis and diseases associated therewith.

The present invention provides a method for treating and preventing cell necrosis and diseases associated therewith, comprising inhibiting the expression, activation and/or enzymatic activity of intracellular elastase, or by over expression of one or more endogenous inhibitors of said intracellular elastase, or by upregulation of the expression of said endogenous inhibitors.

In one aspect, the abovementioned method comprises administering to a subject a therapeutically effective amount of one or more elastase inhibiting agents, wherein said agents inhibit the enzymatic activity of intracellular elastase in the cells to be treated.

In another aspect, the method of the invention comprises administering to a subject a therapeutically effective

amount of one or more agents that inhibit the activation of inactive intracellular elastase in the cells to be treated.

In a further aspect, the method of the invention comprises specifically inhibiting or reducing the expression of the gene(s) encoding elastase in the cells to be treated. Any suitable method for silencing or reducing the elastase gene expression may be used in order to achieve this aim. In one preferred embodiment, inhibition of elastase gene expression is accomplished by administering sequence-specific antisense oligonucleotides to the subject in whom necrosis is to be treated or prevented. In another preferred embodiment, inhibition of elastase gene expression is accomplished by administering to the subject ribozymes with sequence specificity for elastase mRNA. In another aspect, the method of the invention comprises increasing the expression of the endogenous inhibitor(s) of elastase.

The present invention also encompasses a method for inhibiting and preventing cell necrosis in vitro, comprising causing an effective amount of one or more elastase inhibitors to enter the cells to be treated.

The inventors have also surprisingly found the inhibition of elastase within the affected cells may shift cell necrosis, at least partially, into apoptotic cell death. Thus, in a preferred embodiment, the invention provides a method for treating and preventing cell necrosis and diseases associated therewith, comprising:  
inhibiting the expression, activation and/or enzymatic activity of intracellular elastase, or by over expression

of one or more endogenous inhibitors of said intracellular elastase, or by upregulation of the expression of said endogenous inhibitors; and inhibiting apoptotic cell death.

The present invention is also directed to pharmaceutical compositions for the treatment and/or prevention of cell necrosis and diseases associated therewith, wherein said compositions comprise one or more agents that inhibit expression, activation and/or enzymatic activity of intracellular elastase, or elevate levels of its endogenous inhibitors.

In one aspect, the abovementioned pharmaceutical compositions comprise one or more elastase inhibitors that are capable of entering the cells to be treated.

In another aspect, the aforementioned pharmaceutical composition of the present invention comprises one or more agents that inhibit the activation of inactive intracellular elastase in the cells to be treated.

In a further aspect, the aforementioned pharmaceutical compositions comprise antisense nucleotides that are capable of entering the cells to be treated, wherein said nucleotides have nucleotide sequences complementary to one or more elastase-specific mRNA strands.

In yet another aspect, the pharmaceutical compositions of the present invention comprise ribozymes that are capable of entering the cells to be treated, wherein said ribozymes bind to one or more elastase-specific mRNA strands,

following which, said mRNA strands are cleaved by said ribozymes.

In a further aspect of the present invention is provided the use of one or more elastase inhibitors in the preparation of a medicament for treating and/or preventing necrosis and diseases associated therewith.

The present invention also provides the use of one or more agents that inhibit the activation of inactive intracellular elastase in the cells to be treated, in the preparation of a medicament for treating and/or preventing necrosis and diseases associated therewith.

The present invention is further directed to the use of one or more antisense nucleotides in the preparation of a medicament for treating and/or preventing necrosis and diseases associated therewith, wherein said nucleotides have nucleotide sequences complementary to one or more elastase-specific mRNA strands

The present invention also provides for the use of ribozymes in the preparation of a medicament for treating and/or preventing necrosis and diseases associated therewith, wherein said ribozymes bind to, and cleave, one or more elastase-specific mRNA strands.

The inhibitors of elastase activity used according to the invention for treating and preventing cell necrosis, and diseases associated therewith, are all capable of entering into the targeted cells, such that said inhibitors exert their inhibitory actions within said cells.



Preferably, necrosis may be treated or prevented according to the present invention in cells selected from the group consisting of neuronal cells, purkinje cell, hyppocampal pyramidal cells, glia cells, cells of hematopoetic origin (such as lymphocyte and macrophage), hepatocytes, thymocytes, fibroblast, myocard cells, epithelial cells, broncho epithelial cells, glomeruli, lung epithelial cells, keratinocytes, gastrointestinal cells, psoriatic epidermis cells, bone and cartilage cells.

Preferably, the diseases associated with cell necrosis, which may be treated and/or prevented according to the present invention, are selected from the group consisting of neurodegenerative disorders (e.g., dementia), leukemias, lymphomas, neonatal respiratory distress, asphyxia, incarcerated hernia, diabetes mellitus, tuberculosis, endometriosis, vascular dystrophy, psoriasis, cold injury, iron-load complications, complications of steroid treatment, ischemic heart disease, reperfusion injury, cerebrovascular disease or damage, gangrene, pressure sores, pancreatitis, hepatitis, bacterial sepsis, viral sepsis, burns, hyperhermia, Crohn's disease, celiac disease, compartment syndrome, necrotizing procolitis and cystic fibrosis, rheumatoid arthritis, nephrotoxicity, multiple sclerosis, spiral cord injury, glomerulonephritis, muscular dystrophy and degenerative arthritis.

#### Brief Description of the Drawings

Fig. 1 graphically depicts the percentage of necrotic and apoptotic cells observed following treatment with and without oligomycin and anti-Fas.

Fig. 2 is a photographic representation of gelatin substrate gel electrophoresis results for lysates of U-937 cells treated/untreated with oligomycin and/or anti-Fas for 3 hours.

Fig. 3 is a photographic representation of gelatin substrate gel electrophoresis results obtained for lysates of U-937 cells treated/untreated with 0.5 mM KCN for 3 hours.

Fig. 4 presents results showing the effect of elastase inhibitor III on staurosporine-induced necrosis/apoptosis in PC-12 cells. The figure diagrammatically depicts the proportion of live, necrotic and apoptotic cells following various treatments. The numerical values for these proportions are given in the accompanying table.

Fig. 5 presents results demonstrating the effect of elastase inhibitor III on KCN-induced necrosis in PC-12 cells. Panel A diagrammatically depicts the proportion of live, necrotic and apoptotic cells following various treatments. The numerical values for these proportions are given in the accompanying table. Panel B graphically depicts percentage PC-12 cell survival following treatment with KCN in the presence/absence of elastase inhibitor III.

Fig. 6 diagrammatically depicts the proportion of live, necrotic and apoptotic U-937 cells following treatment with KCN in the presence/absence of elastase inhibitor III. The numerical values for these proportions are given in the accompanying table.

**Detailed Description of Preferred Embodiments**

The term "necrosis", as used herein, encompasses cell necrosis states, as well as intermediates states, exhibiting necrotic and apoptotic characteristics. The term "elastase", as used herein, refers to one or more forms of said enzyme.

Compounds exhibiting elastase inhibitory profile, which are herein referred to as elastase inhibiting agents, or elastase inhibitors, are known in the art, and are disclosed, for example, by Stein et. al. [Biochemistry 25, p. 5414 (1986)], Powers et al. [Biochim. Biophys. Acta. 485, p. 15 (1977)], US 4,683,241, US 5,216,022, and US 6,159,938. Inhibitors of elastase are also commercially available from, e.g., Sigma-Aldrich or Calbiochem-Novabiochem Corporation.

Elastase inhibitors used according to the present invention are formulated together with one or more pharmaceutically acceptable carriers, which are non-toxic, inert solid, semi-solid or liquid fillers, diluent, encapsulating material or formulation auxiliary of any type. The pharmaceutical compositions can be administered to human and other mammalian subjects in any acceptable route, and preferably orally, parenterally or topically.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or fillers or extenders such as starches, lactose, sucrose, glucose and mannitol, binders such as carboxymethylcellulose and gelatin, humectants such as glycerol, disintegrating agents such as agar-agar, calcium

carbonate and potato starch, absorbents and lubricants. The solid dosage forms can be prepared with coatings and shells according to methods known in the art.

Liquid dosage forms for oral administration include pharmaceutically acceptable solutions, emulsions, suspensions and syrups. In addition to the active compounds, the liquid dosage form may contain inert diluents commonly used in the art such as water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, propylene glycol and oils. Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Injectable preparations suitable for parenteral administration are provided in the form of pharmaceutically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions prior to use. Examples of suitable aqueous or non-aqueous carriers or vehicles include water, Ringer's solution and isotonic sodium chloride solution. Sterile oils may also be employed as a suitable suspending medium. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents therein.

Dosage forms for topical or transmucosal administration of elastase inhibitors according to the invention may include pastes, creams, lotions, gels, powders, solutions and

sparys. In addition to the active ingredient, the pastes, creams and gels may contain excipients such as fats, oils, waxes, paraffins, strach, cellulose derivatives, polyethylene glycols, talc, zinc oxide, or mixture thereof. Powders and sprays can contain excipient such as lactose, tals, silicic acid, aluminum hydroxide, calcium silicats and mixtures thereof.

Other suitable formulations may be prepared by encapsulating the active ingredient in lipid vesicles or in biodegradable polymeric matrices, or by attaching said active ingredient to monoclonal antibodies. Methods to form liposomes are known in the art.

Dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the elastase inhibitor that is effective to achieve the desired therapeutic response for a particular patient. The selected dosage form will depend on the activity of the particular elastase inhibitor, the route of administration, the severity of the condition being treated and other factors associated with the patient being treated. Typical dose regimes are in the range of 0.1-200 mg/kg.

In one aspect, the present invention is directed to a method for treating or preventing cell necrosis by means of inhibiting or silencing expression of the gene or genes that encode intracellular elastase. Several techniques for reducing or blocking gene expression have been developed; these techniques are all included within the scope of the present invention.

One example of this type of technique is the use of a sequence-specific antisense oligonucleotide in order to bind and inactivate the mRNA sequences responsible for elastase expression. Such antisense oligonucleotides may be administered on a continuous basis, by means of their in vivo production following transfection of target cells with an expression vector encoding the desired antisense sequence. Alternatively, preformed antisense oligonucleotides may be administered parenterally, or in certain circumstances, orally. Pharmaceutical compositions comprising antisense oligonucleotides may be formulated as described hereinabove.

In another approach, double-stranded oligonucleotides that attach to DNA binding proteins may be used to prevent the activation of the elastase genes.

A third approach involves the use of ribozymes. Ribozymes are natural RNA sequences that bind and cleave specific RNA molecules. Following modification of such a sequence, a ribozyme with specificity for elastase gene(s) could be developed.

In another aspect, the present invention is directed to a method for treating or preventing cell necrosis by means of over expression of one or more endogenous inhibitors of said intracellular elastase, or by upregulation of the expression of said endogenous inhibitors. Several useful techniques are described in *Molecular Cloning - a Laboratory Manual*, by Sambrook, Fritsch and Maniatis (Coldstring Harbor Laboratory Press, 1989).

In another aspect, the present invention is directed to the treatment or prevention of cell necrosis by means of inhibiting the expression, activation and/or enzymatic activity of intracellular elastase, or by over expression of one or more endogenous inhibitors of said intracellular elastase, or by upregulation of the expression of said endogenous inhibitors and, in addition, inhibiting apoptotic cell death. In a preferred embodiment of this aspect of the invention, the inhibition of apoptotic cell death is accomplished by means of administering to subject a therapeutic effective amount of an anti-apoptotic agent, which is preferably selected from the group consisting of [R]-N-[2-heptyl]-methylpropargylamine (R-2HMP), vitamin E, vitamin D and the hydrophilic bile salt ursodeoxycholic acid. Other methods known in the art for inhibiting apoptosis, for example, by means of regulation of expression of pro- and anti- apoptotic proteins, may also be used according to the present invention. Such methods are described, for example, by Li et al. [Acta. Anaesthesiol Sin, 38(4), p. 207-215 (2000)].

### Examples

#### Experimental protocol

##### 1. Models of necrosis in vitro

##### Staurosporine and anti-Fas-induced necrosis

Human promonocytic U-937 cells in logarithmic phase were seeded at a concentration of  $4 \times 10^5$ /ml. Afterwards the cells were washed twice and seeded again in glucose-free RPMI-1640 medium (Beit Haemek, Israel) supplemented with 2 mM pyruvate (Beit Haemek, Israel) and 10% dialyzed FCS (Gibco, BRL) for one hour.

The rat pheochromocytoma PC-12 cell line was propagated in DMEM medium (Gibco, BRL), supplemented with 5% heat-inactivated calf serum, 10% heat-inactivated horse serum, and 2 mM L-glutamine. PC-12 cells in logarithmic phase were seeded at a concentration of  $1.2 \times 10^5$ /well in 24-well plates (Cellstar). Then the cells were washed twice and maintained in glucose-free RPMI-1640 medium (Beit Haemek, Israel), and supplemented with 2 mM pyruvate and 10% dialyzed FCS for one hour. U-937 and PC-12 cells were incubated with and without 1  $\mu$ M oligomycin (Sigma) for 45 min, and cells were treated with or without 1.25  $\mu$ M staurosporine (STS) (Sigma) for an additional seven hours in U-937 cells or five hours in PC-12 cells. Alternatively, cells were treated with or without 100 ng/ml anti-Fas (Upstate biotechnology, USA) for the same time period.

#### KCN-induced necrosis

U-937 and PC-12 cells cultured in complete RPMI-1640 medium were washed and seeded in glucose-free RPMI-1640 medium, as described above, and treated with or without 0.5 mM KCN (Merck, Germany) for seven hours with U-937 cells, or for five hours with PC-12 cells.

#### 2. Testing of elastase inhibitor

200  $\mu$ M elastase inhibitor III (MeOSuc-Ala-Ala-Pro-Val-CMK from Calbiochem) when added was administered 30 min before addition of the inducers. The inhibitor was dissolved in DMSO to a concentration of 100 mM. The final concentration of DMSO in the system was 0.2%, and was added to all treatments.



### 3. Cell death assay

#### Trypan blue exclusion

At each time point, cell viability was determined by the trypan blue exclusion method (Daniel CP, Parreira A., et al. Leukemia Res. 11:191-196 (1987)). Assays were performed in duplicate.

#### Morphological quantification of apoptosis and necrosis

Cells undergoing morphological changes associated with apoptotic or necrotic cell death were monitored as described by McGahon et al. [Methods Cell Biol, 46: p. 153-85 (1995)]. Briefly, 1 ml of the cells was collected and centrifuged. The pellet was resuspended in a 20-fold dilution of the dye mixture (composed of 100 mg/ml acridine orange and 100 mg/ml ethidium bromide in PBS), placed on a glass slide and viewed on an inverted fluorescence microscope. A minimum of 200 cells was scored for each sample.

#### Preparation of cell lysates

$4 \times 10^7$  U-937 cells, treated or untreated with the various inducers, were collected after three hours of incubation, washed twice with ice-cold PBS and resuspended at  $10^8$ /ml in ice-cold lysing buffer (50 mM Tris-HCl pH 7.5, 0.1 % NP-40, 1 mM DTT, 100  $\mu$ M leupeptin and 100  $\mu$ M TLCK). The cells were broken by the use of a polytron device (4 cycles of 7 seconds each) on ice, and the debris was pelleted by centrifugation in an ultracentrifuge at 120,000 x g for 30 minutes at 4°C. The supernatant was used immediately or stored at -70°C. The protein content of each sample was determined by the BCA assay (BioRad).

### 5. Electrophoresis

Electrophoresis on a gelatin substrate gel was performed as previously described (Distefano J. F., Cotto C. A., et al. Cancer Invest. 6, 487-498, (1988)). Proteases were reversibly inactivated by addition of 100  $\mu$ l aliquots of the cell lysates containing 200  $\mu$ g protein to 50  $\mu$ l of 0.625 M Tris-HCl buffer, pH 6.8, with 2.5% SDS, 10% sucrose and 0.03% phenol red. Samples were then electrophorated using 0.1% gelatin copolymerized in 11% polyacrylamide gel. After electrophoresis, the gels were subjected to three repeated immersions in 0.1 M Tris-HCl buffer, pH 7.0, containing 2.5% (V/V) Triton-x-100 in order to remove the SDS and reactivate the proteases. The gels were sliced and incubated overnight at 37° C in 0.1 M glycine-NaOH buffer, pH 7.0, with or without 100  $\mu$ M TPCK (chymotrypsin-like serine protease inhibitor) and 100  $\mu$ M elastinal (elastase-like serine protease inhibitor). The bands of protease activity were developed with amido black staining.

### Results

#### 1. Anti-Fas-induced apoptosis/necrosis in U-937 cells

Fig. 1 indicates that treatment with anti-Fas induced about 60% apoptosis as compared to the control. Oligomycin is inactive by itself, however, addition of anti-Fas to oligomycin switched apoptotic cell death to necrotic cell death. Under these conditions, about 70% necrosis occurred and apoptosis returned to control level. Nuclear morphology was determined and analyzed by fluorescence microscope after double-staining with acridine orange and ethidium bromide.

## 2. Induction of elastase-like activity during necrotic cell death induced by anti-Fas in the presence of oligomycin

U-937 cells were maintained in glucose-free medium preincubated with or without 1  $\mu$ M oligomycin for 45 min and treated with or without 100 ng/ml anti-Fas for three hours. Following this, cell lysates were prepared as described in "Experimental protocol" and applied to a gelatine substrate gel electrophoresis. The results, which are presented in Fig. 2 indicate that treatment with anti-Fas and oligomycin caused the appearance of a band of protease activity (line D), which was not found in the untreated control cells (lane A), anti-Fas-treated cells (lane B), or oligomycin-treated cells (lane C). This band disappeared in the presence of 100  $\mu$ M elastinal (lane D), but not in the presence of 100  $\mu$ M TPCK (lane D), indicating that treatment with anti-Fas and oligomycin induced an elastase-like activity, but not a chymotrypsin-like activity.

## 3. Induction of elastase-like activity during necrotic cell death induced by KCN

U-937 cells were treated with or without 0.5 mM KCN for three hours and then cell lysates were prepared as described in "Experimental protocol" and applied to a gelatine substrate gel electrophoresis. The results, which are presented in Fig. 3, show that treatment with KCN caused the appearance of a band of protease activity (lane B), which was not found in the untreated control cells (lane A). This band disappeared in the presence of 100  $\mu$ M elastinal (lane B), but not in the presence of 100  $\mu$ M TPCK (lane B), indicating that treatment with KCN induced an elastase-like activity, but not a chymotrypsin-like activity.

4. Elastase inhibitor III prevents STS-induced necrosis/apoptosis in PC-12 cells

Fig. 4 indicates that treatment with STS induced 70% apoptosis as compared to 2% in the control. Oligomycin is inactive by itself, however, addition of STS to oligomycin switched apoptotic cell death to necrotic cell death. Under these conditions, 38% necrosis occurred and apoptosis returned to control level. Elastase inhibitor III inhibited necrosis induced by STS and oligomycin to 16%. Apoptosis induced by STS was reduced from 70% to 28%.

5. Prevention of KCN-induced necrosis by elastase inhibitor III in PC-12 cells

Exposure of PC-12 cells to KCN induced massive necrotic cell death compared to the control. Addition of elastase inhibitor III which was inactive by itself significantly inhibited necrosis induced by KCN (Fig. 5, A). The protective effect of elastase inhibitor III is also seen when cell survival was determined under the same conditions by trypan blue exclusion (Fig. 5, B).

6. Inhibitory effect of elastase inhibitor III on KCN-induced necrosis in U-937 cells

Treatment with KCN caused 95% necrosis as compared to 10% in the control. Addition of elastase inhibitor III with KCN markedly reduced necrotic cell death to 21%, and shifted 22% of the necrotic cell death to apoptotic cell death. 52% of the cells were protected from necrotic cell death by this inhibitor. Elastase inhibitor III did not cause any cell damage (Fig. 6).

**Claims**

1. A method for treating and/or preventing cell necrosis and diseases associated therewith, comprising the inhibition of intracellular elastase.
2. A method according to claim 1, wherein the inhibition of intracellular elastase is carried out by inhibiting the expression, activation and/or enzymatic activity of said intracellular elastase, or by over expression of one or more endogenous inhibitors of said intracellular elastase, or by upregulation of the expression of said endogenous inhibitors.
3. A method according to claim 2, comprising administering to a subject a therapeutically effective amount of one or more elastase inhibiting agents, wherein said agents inhibit the enzymatic activity of intracellular elastase in the cells to be treated.
4. A method according to claim 2, comprising administering to a subject a therapeutically effective amount of one or more agents that inhibit the activation of inactive intracellular elastase in the cells to be treated.
5. A method according to claim 2, comprising specifically inhibiting or reducing the expression of the gene(s) encoding elastase in the cells to be treated.
6. A method according to claim 5, comprising administering to a subject a therapeutically effective amount of sequence-specific antisense oligonucleotides.

7. A method according to claim 5, comprising administering to a subject a therapeutically effective amount of ribozymes with sequence specificity for elastase mRNA.

8. A method according to any one of claims 1-7, which further comprises inhibiting apoptotic cell death.

9. A method for inhibiting and preventing cell necrosis *in vitro*, comprising causing an effective amount of one or more elastase inhibitors to enter the cells to be treated.

10. Pharmaceutical compositions for the treatment and/or prevention of cell necrosis and diseases associated therewith, wherein said compositions comprise one or more agents that inhibit expression, activation and/or enzymatic activity of intracellular elastase, or elevate levels of its endogenous inhibitors, and a pharmaceutically acceptable carrier.

11. Pharmaceutical compositions according to claim 10, comprising one or more elastase inhibitors that are capable of entering the cells to be treated.

12. Pharmaceutical compositions according to claim 10, comprising one or more agents that inhibit the activation of inactive intracellular elastase in the cells to be treated.

13. Pharmaceutical compositions according to claim 10, comprising antisense nucleotides that are capable of entering the cells to be treated, wherein said nucleotides

have nucleotide sequences complementary to one or more elastase-specific mRNA strands.

14. Pharmaceutical compositions according to claim 10, comprising ribozymes that are capable of entering the cells to be treated, wherein said ribozymes bind to one or more elastase-specific mRNA strands, following which, said mRNA strands are cleaved by said ribozymes.

15. A method according to claim 1, wherein the cells to be treated are selected from the group consisting neuronal cells, purkinje cell, hyppocampal pyramidal cells, glia cells, hematopoetic cells, Lymphocyte, macrophage, hepatocytes, thymocytes, muscle cells, fibroblast, myocard cells, epithelial cells, broncho epithelial cells, glomeruli, lung epithelial cells, keratinocytes, gastrointestinal cells, psoriatic epidermis cells, bone and cartilage cells.

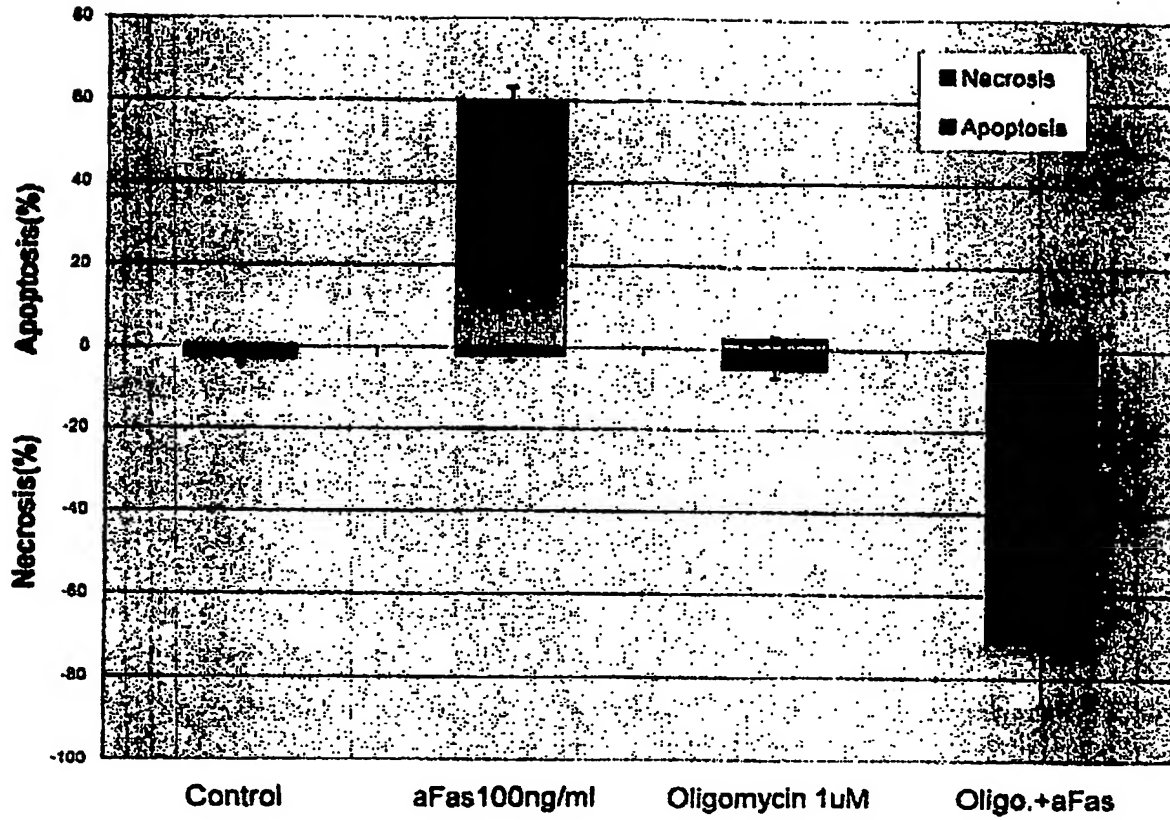
16. A method according to claim 1, wherein the diseases associated with cell necrosis are selected from the group consisting of neurodegenerative disorders, leukemias, lymphomas, neonatal respiratory distress, asphyxia, incarcerated hernia, diabetes mellitus, tuberculosis, endometriosis, vascular dystrophy, psoriasis, cold injury, iron-load complications, complications of steroid treatment, ischemic heart disease, reperfusion injury, cerebrovascular disease or damage, gangrene, pressure sores, pancreatitis, hepatitis, bacterial sepsis, viral sepsis, burns, hyperthermia, Crohn's disease, celiac disease, compartment syndrome, necrotizing procolitis, cystic fibrosis, rheumatoid arthritis, nephrotoxicity,

multiple sclerosis, spiral cord injury, glomerulonephritis, muscular dystrophy and degenerative arthritis.

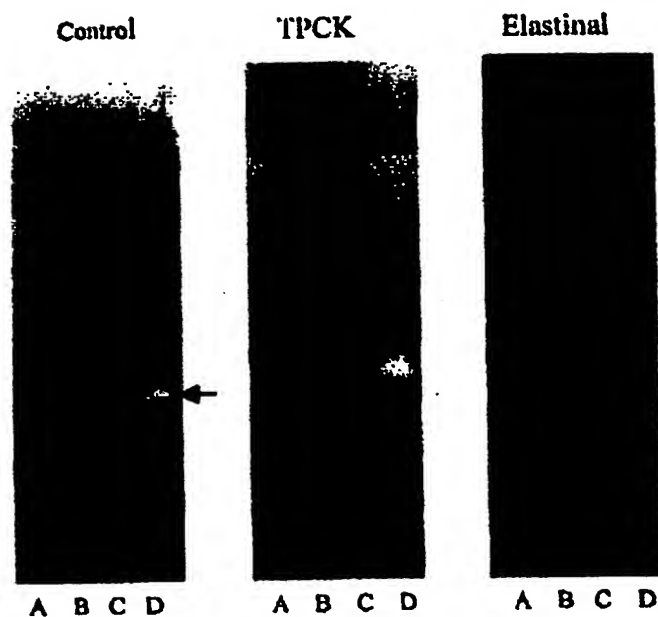
17. A pharmaceutical composition according to claim 10, for the treatment and/or prevention of cell necrosis in cells selected from the group consisting of neuronal cells, purkinje cell, hyppocampal pyramidal cells, glia cells, hematopoetic cells, Lymphocyte, macrophage, hepatocytes, thymocytes, muscle cells, fibroblast, myocard cells, epithelial cells, broncho epithelial cells, glomeruli, lung epithelial cells, keratinocytes, gastrointestinal cells, psoriatic epidermis cells, bone and cartilage cells.

18. A pharmaceutical composition according to claim 10, wherein the diseases associated with cell necrosis are selected from the group consisting of neurodegenerative disorders, leukemias, lymphomas, neonatal respiratory distress, asphyxia, incarcerated hernia, diabetes mellitus, tuberculosis, endometriosis, vascular dystrophy, psoriasis, cold injury, iron-load complications, complications of steroid treatment, ischemic heart disease, reperfusion injury, cerebrovascular disease or damage, gangrene, pressure sores, pancreatitis, hepatitis, bacterial sepsis, viral sepsis, burns, hyperthermia, Crohn's disease, celiac disease, compartment syndrome, necrotizing procolitis, cystic fibrosis, rheumatoid arthritis, nephrotoxicity, multiple sclerosis, spiral cord injury, glomerulonephritis, muscular dystrophy and degenerative arthritis.

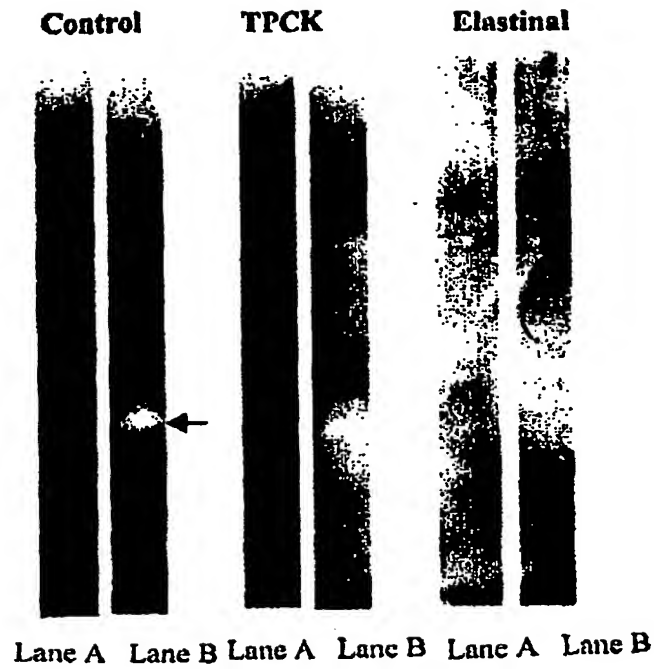




**Fig. 1**



**Fig. 2**



**Fig. 3**

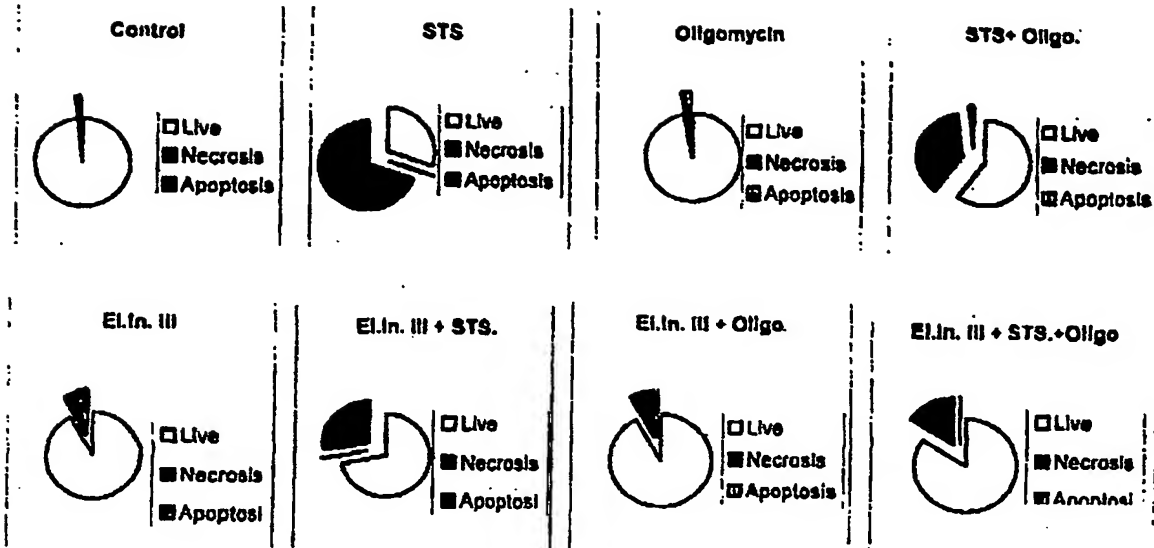
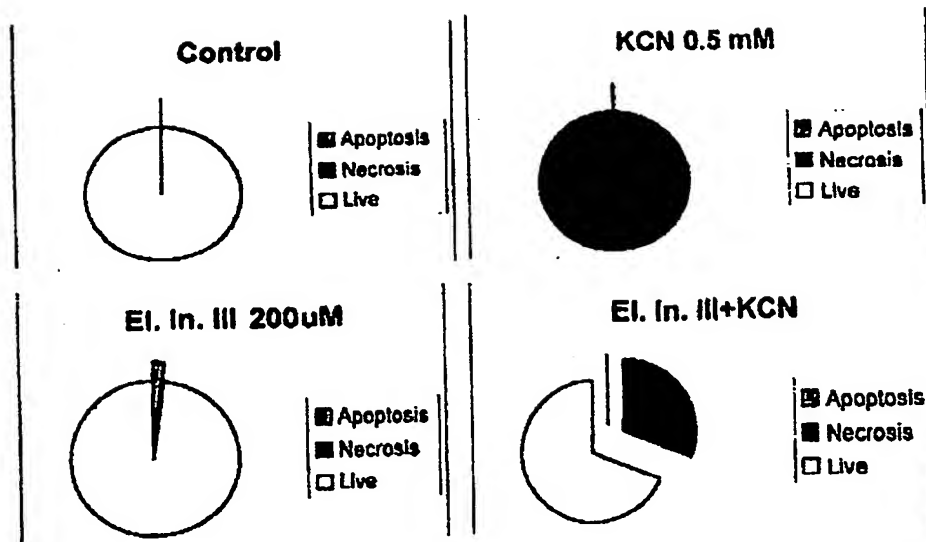


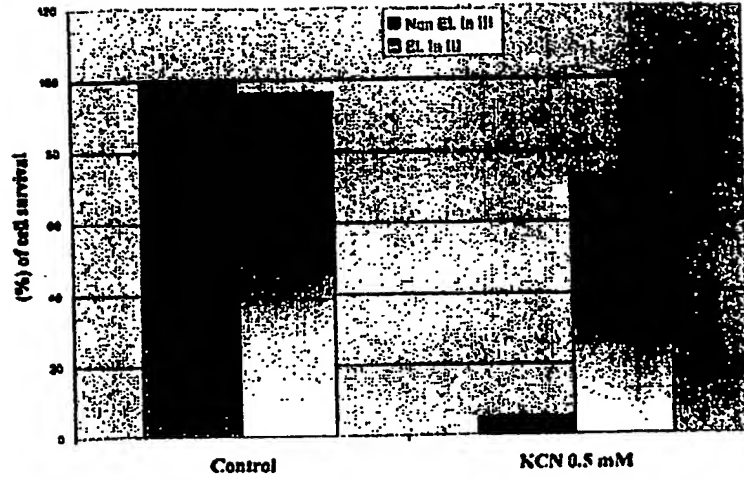
Fig. 4

|                           | Live | Necrosis | Apoptosis |
|---------------------------|------|----------|-----------|
| Control                   | 98%  |          | 2%        |
| Staurosporine(STS) 1.25uM | 30%  |          | 70%       |
| Oligomycin 1uM            | 97%  | 1%       | 2%        |
| STS+ Oligo.               | 60%  | 38%      | 2%        |
| El.In. III 200uM          | 92%  | 3%       | 5%        |
| El.In. III + Oligo.       | 92%  | 8%       |           |
| El.In. III + STS.         | 72%  |          | 28%       |
| El.In. III + STS.+Oligo   | 84%  | 16%      |           |

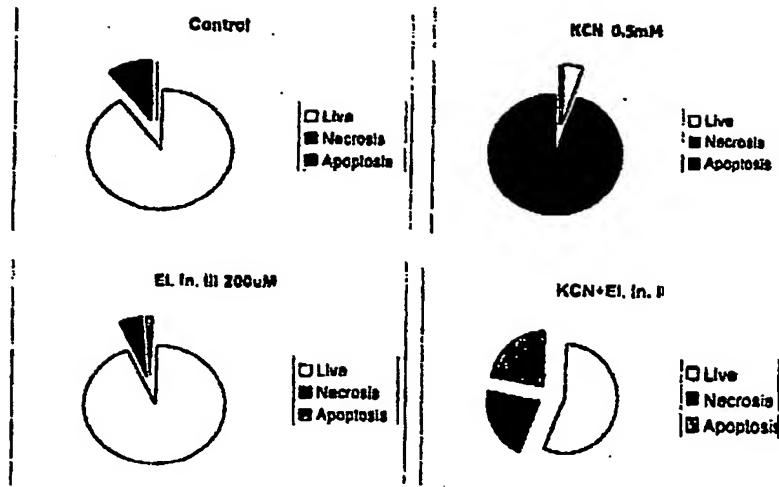


|                   | Live | Necrosis | Apoptosis |
|-------------------|------|----------|-----------|
| Control           | 100% | 0%       | 0%        |
| KCN 0.5mM         | 0%   | 100%     | 0%        |
| El. In. III 200uM | 98%  | 2%       | 0%        |
| KCN+El. In. III   | 69%  | 31%      | 0%        |

**Fig. 5 A**



**Fig. 5 B**



|                   | Live | Necrosis | Apoptosis |
|-------------------|------|----------|-----------|
| Con               | 90%  | 10%      |           |
| KCN 0.5mM         | 5%   | 95%      |           |
| EI. In. III 200uM | 93%  | 5.5%     | 1.5%      |
| KCN+EI. In. III   | 57%  | 21%      | 22%       |

**Fig. 6**

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